

1 INDIVIDUALIZED ANTI-CANCER ANTIBODIES

2 REFERENCE TO RELATED APPLICATIONS:

3 This application is a divisional of application S.N.
4 09/727,361, filed November 29,2000, which is a continuation-
5 in-part of application S.N. 09/415,278, filed October 8,
6 1999, now U.S. Patent 6,180,357, the contents of each are
7 herein incorporated by reference.

8 FIELD OF THE INVENTION:

9 This invention relates to the production of anti-cancer
10 antibodies customized for the individual patient which may be
11 combined with chemotherapeutic agents that can be used for
12 therapeutic and diagnostic purposes. The invention further
13 relates to the process by which the antibodies are made and
14 to their methods of use.

15 BACKGROUND OF THE INVENTION:

16 Each individual who presents with cancer is unique and
17 has a cancer that is as different from other cancers as that
18 person's identity. Despite this, current therapy treats all
19 patients with the same type of cancer, at the same stage, in
20 the same way. At least 30% of these patients will fail the
21 first line therapy, thus leading to further rounds of
22 treatment and the increased probability of treatment failure,
23 metastases, and ultimately, death. A superior approach to
24 treatment would be the customization of therapy for the
25 particular individual. The only current therapy which lends

1 itself to customization is surgery. Chemotherapy and
2 radiation treatment can not be tailored to the patient, and
3 surgery by itself, in most cases is inadequate for producing
4 cures.

5 With the advent of monoclonal antibodies, the
6 possibility of developing methods for customized therapy
7 became more realistic since each antibody can be directed to
8 a single epitope. Furthermore, it is possible to produce a
9 combination of antibodies that are directed to the
10 constellation of epitopes that uniquely define a particular
11 individual's tumor.

12 Having recognized that a significant difference between
13 cancerous and normal cells is that cancerous cells contain
14 antigens that are specific to transformed cells, the
15 scientific community has long held that monoclonal antibodies
16 can be designed to specifically target transformed cells by
17 binding specifically to these cancer antigens; thus giving
18 rise to the belief that monoclonal antibodies can serve as
19 "Magic Bullets" to eliminate cancer cells.

20 At the present time, however, the cancer patient usually
21 has few options of treatment. The regimented approach to
22 cancer therapy has produced improvements in global survival
23 and morbidity rates. However, to the particular individual,
24 these improved statistics do not necessarily correlate with
25 an improvement in their personal situation.

1 Thus, if a methodology was put forth which enabled the
2 practitioner to treat each tumor independently of other
3 patients in the same cohort, this would permit the unique
4 approach of tailoring therapy to just that one person. Such
5 a course of therapy would, ideally, increase the rate of
6 cures, and produce better outcomes, thereby satisfying a
7 long-felt need.

8 Historically, the use of polyclonal antibodies has been
9 used with limited success in the treatment of human cancers.
10 Lymphomas and leukemias have been treated with human plasma,
11 but there were few prolonged remission or responses.
12 Furthermore, there was a lack of reproducibility and there
13 was no additional benefit compared to chemotherapy. Solid
14 tumors such as breast cancers, melanomas and renal cell
15 carcinomas have also been treated with human blood,
16 chimpanzee serum, human plasma and horse serum with
17 correspondingly unpredictable and ineffective results.

18 There have been many clinical trials of monoclonal
19 antibodies for solid tumors. In the 1980s there were at least
20 four clinical trials for human breast cancer which produced
21 only one responder from at least 47 patients using antibodies
22 against specific antigens or based on tissue selectivity. It
23 was not until 1998 that there was a successful clinical trial
24 using a humanized anti-her 2 antibody in combination with
25 Cisplatin. In this trial 37 patients were accessed for

1 responses of which about a quarter had a partial response
2 rate and another half had minor or stable disease
3 progression.

4 The clinical trials investigating colorectal cancer
5 involve antibodies against both glycoprotein and glycolipid
6 targets. Antibodies such as 17-1A, which has some
7 specificity for adenocarcinomas, had undergone Phase 2
8 clinical trials in over 60 patients with only one patient
9 having a partial response. In other trials, use of 17-1A
10 produced only one complete response and two minor responses
11 among 52 patients in protocols using additional
12 cyclophosphamide. Other trials involving 17-1A yielded
13 results that were similar. The use of a humanized murine
14 monoclonal antibody initially approved for imaging also did
15 not produce tumor regression. To date there has not been an
16 antibody that has been effective for colorectal cancer.
17 Likewise there have been equally poor results for lung
18 cancer, brain cancers, ovarian cancers, pancreatic cancer,
19 prostate cancer, and stomach cancer. There has been some
20 limited success in the use of anti-GD3 monoclonal antibody
21 for melanoma. Thus, it can be seen that despite successful
22 small animal studies that are a prerequisite for human
23 clinical trials, the antibodies that have been tested have
24 been for the most part ineffective.

1 PRIOR PATENTS:

2 U.S. Patent No. 5,750,102 discloses a process wherein
3 cells from a patient's tumor are transfected with MHC genes
4 which may be cloned from cells or tissue from the patient.
5 These transfected cells are then used to vaccinate the
6 patient.

7 U.S. Patent No. 4,861,581 discloses a process comprising
8 the steps of obtaining monoclonal antibodies that are
9 specific to an internal cellular component of neoplastic and
10 normal cells of the mammal but not to external components,
11 labeling the monoclonal antibody, contacting the labeled
12 antibody with tissue of a mammal that has received therapy to
13 kill neoplastic cells, and determining the effectiveness of
14 therapy by measuring the binding of the labeled antibody to
15 the internal cellular component of the degenerating
16 neoplastic cells. In preparing antibodies directed to human
17 intracellular antigens, the patentee recognizes that
18 malignant cells represent a convenient source of such
19 antigens.

20 U.S. Patent No. 5,171,665 provides a novel antibody and
21 method for its production. Specifically, the patent teaches
22 formation of a monoclonal antibody which has the property of
23 binding strongly to a protein antigen associated with human
24 tumors, e.g. those of the colon and lung, while binding to
25 normal cells to a much lesser degree.

1 U.S. Patent No. 5,484,596 provides a method of cancer
2 therapy comprising surgically removing tumor tissue from a
3 human cancer patient, treating the tumor tissue to obtain
4 tumor cells, irradiating the tumor cells to be viable but
5 non-tumorigenic, and using these cells to prepare a vaccine
6 for the patient capable of inhibiting recurrence of the
7 primary tumor while simultaneously inhibiting metastases.
8 The patent teaches the development of monoclonal antibodies
9 which are reactive with surface antigens of tumor cells. As
10 set forth at col. 4, lines 45 et seq., the patentees utilize
11 autochthonous tumor cells in the development of monoclonal
12 antibodies expressing active specific immunotherapy in human
13 neoplasia.

14 U.S. Patent No. 5,693,763 teaches a glycoprotein antigen
15 characteristic of human carcinomas and not dependent upon the
16 epithelial tissue of origin.

17 U.S. Patent No. 5,783,186 is drawn to Anti-Her2
18 antibodies which induce apoptosis in Her2 expressing cells,
19 hybridoma cell lines producing the antibodies, methods of
20 treating cancer using the antibodies and pharmaceutical
21 compositions including said antibodies.

22 U.S. Patent No. 5,849,876 describes new hybridoma cell
23 lines for the production of monoclonal antibodies to mucin
24 antigens purified from tumor and non-tumor tissue sources.

1 U.S. Patent No. 5,869,268 is drawn to a method for
2 producing a human lymphocyte producing an antibody specific
3 to a desired antigen, a method for producing a monoclonal
4 antibody, as well as monoclonal antibodies produced by the
5 method. The patent is particularly drawn to the production
6 of an anti-HD human monoclonal antibody useful for the
7 diagnosis and treatment of cancers.

8 U.S. Patent No. 5,869,045 relates to antibodies,
9 antibody fragments, antibody conjugates and single chain
10 immunotoxins reactive with human carcinoma cells. The
11 mechanism by which these antibodies function is two-fold, in
12 that the molecules are reactive with cell membrane antigens
13 present on the surface of human carcinomas, and further in
14 that the antibodies have the ability to internalize within
15 the carcinoma cells, subsequent to binding, making them
16 especially useful for forming antibody-drug and antibody-
17 toxin conjugates. In their unmodified form the antibodies
18 also manifest cytotoxic properties at specific
19 concentrations.

20 U.S. Patent No. 5,780,033 discloses the use of
21 autoantibodies for tumor therapy and prophylaxis. However,
22 this antibody is an antinuclear autoantibody from an aged
23 mammal. In this case, the autoantibody is said to be one type
24 of natural antibody found in the immune system. Because the
25 autoantibody comes from "an aged mammal", there is no

1 requirement that the autoantibody actually comes from the
2 patient being treated. In addition the patent discloses
3 natural and monoclonal antinuclear autoantibody from an aged
4 mammal, and a hybridoma cell line producing a monoclonal
5 antinuclear autoantibody.

6 SUMMARY OF THE INVENTION:

7 This application teaches a method for producing patient
8 specific anti-cancer antibodies using a novel paradigm of
9 screening. These antibodies can be made specifically for one
10 tumor and thus make possible the customization of cancer
11 therapy. Within the context of this application, anti-cancer
12 antibodies having either cell-killing (cytotoxic) or cell-
13 growth inhibiting (cytostatic) properties will hereafter be
14 referred to as cytotoxic. These antibodies can be used in
15 aid of staging and diagnosis of a cancer, and can be used to
16 treat tumor metastases.

17 The prospect of individualized anti-cancer treatment
18 will bring about a change in the way a patient is managed. A
19 likely clinical scenario is that a tumor sample is obtained
20 at the time of presentation, and banked. From this sample,
21 the tumor can be typed from a panel of pre-existing anti-
22 cancer antibodies. The patient will be conventionally staged
23 but the available antibodies can be of use in further staging
24 the patient. The patient can be treated immediately with the
25 existing antibodies, and a panel of antibodies specific to

1 the tumor can be produced either using the methods outlined
2 herein or through the use of phage display libraries in
3 conjunction with the screening methods herein disclosed. All
4 the antibodies generated will be added to the library of
5 anti-cancer antibodies since there is a possibility that
6 other tumors can bear some of the same epitopes as the one
7 that is being treated.

8 In addition to anti-cancer antibodies, the patient can
9 elect to receive the currently recommended therapies as part
10 of a multi-modal regimen of treatment. The fact that the
11 antibodies isolated via the present methodology are
12 relatively non-toxic to non-cancerous cells allow
13 combinations of antibodies at high doses to be used, either
14 alone, or in conjunction with conventional therapy. The high
15 therapeutic index will also permit re-treatment on a short
16 time scale that should decrease the likelihood of emergence
17 of treatment resistant cells.

18 If the patient is refractory to the initial course of
19 therapy or metastases develop, the process of generating
20 specific antibodies to the tumor can be repeated for re-
21 treatment. Furthermore, the anti-cancer antibodies can be
22 conjugated to red blood cells obtained from that patient and
23 re-infused for treatment of metastases. There have been few
24 effective treatments for metastatic cancer and metastases
25 usually portend a poor outcome resulting in death. However,

1 metastatic cancers are usually well vascularized and the
2 delivery of anti-cancer antibodies by red blood cells can
3 have the effect of concentrating the antibodies at the site
4 of the tumor. Even prior to metastases, most cancer cells
5 are dependent on the host's blood supply for their survival
6 and anti-cancer antibody conjugated red blood cells can be
7 effective against *in situ* tumors, too. Alternatively, the
8 antibodies may be conjugated to other hematogenous cells,
9 e.g. lymphocytes, macrophages, monocytes, natural killer
10 cells, etc.

11 There are five classes of antibodies and each is
12 associated with a function that is conferred by its heavy
13 chain. It is generally thought that cancer cell killing by
14 naked antibodies are mediated either through antibody
15 dependent cellular cytotoxicity or complement dependent
16 cytotoxicity. For example murine IgM and IgG2a antibodies
17 can activate human complement by binding the C-1 component of
18 the complement system thereby activating the classical
19 pathway of complement activation which can lead to tumor
20 lysis. For human antibodies the most effective complement
21 activating antibodies are generally IgM and IgG1. Murine
22 antibodies of the IgG2a and IgG3 isotype are effective at
23 recruiting cytotoxic cells that have Fc receptors which will
24 lead to cell killing by monocytes, macrophages, granulocytes

1 and certain lymphocytes. Human antibodies of both the IgG1
2 and IgG3 isotype mediate ADCC.

3 Another possible mechanism of antibody mediated cancer
4 killing may be through the use of antibodies that function to
5 catalyze the hydrolysis of various chemical bonds in the cell
6 membrane and its associated glycoproteins or glycolipids, so-
7 called catalytic antibodies.

8 There are two additional mechanisms of antibody mediated
9 cancer cell killing which are more widely accepted. The
10 first is the use of antibodies as a vaccine to induce the
11 body to produce an immune response against the putative
12 cancer antigen that resides on the tumor cell. The second is
13 the use of antibodies to target growth receptors and
14 interfere with their function or to down regulate that
15 receptor so that effectively its function is lost.

16 Accordingly, it is an objective of the invention to
17 teach a method for producing anti-cancer antibodies from
18 cells derived from a particular individual which are
19 cytotoxic with respect to cancer cells while simultaneously
20 being relatively non-toxic to non-cancerous cells.

21 It is an additional objective of the invention to
22 produce novel anti-cancer antibodies.

23 It is a further objective of the instant invention to
24 produce anti-cancer antibodies whose cytotoxicity is mediated
25 through antibody dependent cellular toxicity.

1 It is yet an additional objective of the instant
2 invention to produce anti-cancer antibodies whose
3 cytotoxicity is mediated through complement dependent
4 cellular toxicity.

5 It is still a further objective of the instant invention
6 to produce anti-cancer antibodies whose cytotoxicity is a
7 function of their ability to catalyze hydrolysis of cellular
8 chemical bonds.

9 Still an additional objective of the instant invention
10 is to produce anti-cancer antibodies useful as a vaccine to
11 produce an immune response against putative cancer antigen
12 residing on tumor cells.

13 A further objective of the instant invention is the use
14 of antibodies to target cell membrane proteins, such as
15 growth receptors, cell membrane pumps and cell anchoring
16 proteins, thereby interfering with or down regulating their
17 function.

18 Yet an additional objective of the instant invention is
19 the production of anti-cancer antibodies whose cell-killing
20 utility is concomitant with their ability to effect a
21 conformational change in cellular proteins such that a signal
22 will be transduced to initiate cell-killing.

23 A still further objective of the instant invention is to
24 produce anti-cancer antibodies which are useful for
25 diagnosis, prognosis, and monitoring of cancer, e.g.

1 production of a panel of therapeutic anti-cancer antibodies
2 to test patient samples to determine if they contain any
3 suitable antibodies for therapeutic use.

4 Yet another objective of the instant invention is to
5 produce novel antigens, associated with cancer processes,
6 which can be discovered by using anti-cancer antibodies
7 derived by the process of the instant invention. These
8 antigens are not limited to proteins, as is generally the
9 case with genomic data; they may also be derived from
10 carbohydrates or lipids or combinations thereof.

11 Other objects and advantages of this invention will
12 become apparent from the following description wherein are
13 set forth, by way of illustration and example, certain
14 embodiments of this invention.

15 DETAILED DESCRIPTION OF THE INVENTION:

16 It is to be understood that while a certain form of the
17 invention is illustrated, it is not to be limited to the
18 specific form or arrangement herein described and shown. It
19 will be apparent to those skilled in the art that various
20 changes may be made without departing from the scope of the
21 invention and the invention is not to be considered limited
22 to what is shown and described in the specification.

23 One of the potential benefits of monoclonal antibodies
24 with respect to the treatment of cancer is their ability to
25 specifically recognize single antigens. It was thought that

1 in some instances cancer cells possess antigens that were
2 specific to that kind of transformed cell. It is now more
3 frequently believed that cancer cells have few unique
4 antigens, rather, they tend to over-express a normal antigen
5 or express fetal antigens. Nevertheless, the use of
6 monoclonal antibodies provided a method of delivering
7 reproducible doses of antibodies to the patient with the
8 expectation of better response rates than with polyclonal
9 antibodies.

10 Traditionally, monoclonal antibodies have been made
11 according to fundamental principles laid down by Kohler and
12 Milstein. Mice are immunized with antigens, with or without,
13 adjuvants. The splenocytes are harvested from the spleen for
14 fusion with immortalized hybridoma partners. These are
15 seeded into microtitre plates where they can secrete
16 antibodies into the supernatant that is used for cell
17 culture. To select from the hybridomas that have been plated
18 for the ones that produce antibodies of interest the
19 hybridoma supernatants are usually tested for antibody
20 binding to antigens in an ELISA (enzyme linked immunosorbent
21 assay) assay. The idea is that the wells that contain the
22 hybridoma of interest will contain antibodies that will bind
23 most avidly to the test antigen, usually the immunizing
24 antigen. These wells are then subcloned in limiting dilution
25 fashion to produce monoclonal hybridomas. The selection for
26 the clones of interest is repeated using an ELISA assay to

1 test for antibody binding. Therefore, the principle that has
2 been propagated is that in the production of monoclonal
3 antibodies the hybridomas that produce the most avidly
4 binding antibodies are the ones that are selected from among
5 all the hybridomas that were initially produced. That is to
6 say, the preferred antibody is the one with highest affinity
7 for the antigen of interest.

8 There have been many modifications of this procedure
9 such as using whole cells for immunization. In this method,
10 instead of using purified antigens, entire cells are used for
11 immunization. Another modification is the use of cellular
12 ELISA for screening. In this method instead of using
13 purified antigens as the target in the ELISA, fixed cells are
14 used. In addition to ELISA tests, complement mediated
15 cytotoxicity assays have also been used in the screening
16 process. However, antibody-binding assays were used in
17 conjunction with cytotoxicity tests. Thus, despite many
18 modifications, the process of producing monoclonal antibodies
19 relies on antibody binding to the test antigen as an
20 endpoint.

21 Most antibodies directed against cancer cells have been
22 produced using the traditional methods outlined above. These
23 antibodies have been used both therapeutically and
24 diagnostically. In general, for both these applications, the
25 antibody has been used as the targeting agent that delivers a

1 payload to the site of the cancer. These antibody conjugates
2 can either be radioactive, toxic, or serve as an intermediary
3 for further delivery of a drug to the body, such as an enzyme
4 or biotin. Furthermore, it was widely held, until recently,
5 that naked antibodies had little effect *in vivo*. Both
6 HERCEPTIN and RITUXIMAB are humanized murine monoclonal
7 antibodies that have recently been approved for human use by
8 the FDA. However, both these antibodies were initially made
9 by assaying for antibody binding and their direct
10 cytotoxicity was not the primary goal during the production
11 of hybridomas. Any tendency for these antibodies to produce
12 tumor cell killing is thus through chance, not by design.

13 Although the production of monoclonal antibodies have
14 been carried out using whole cell immunization for various
15 applications the screening of these hybridomas have relied on
16 either putative or identified target antigens or on the
17 selectivity of these hybridomas for specific tissues. It is
18 axiomatic that the best antibodies are the ones with the
19 highest binding constants. This concept originated from the
20 basic biochemical principle that enzymes with the highest
21 binding constants were the ones that were the most effective
22 for catalyzing a reaction. This concept is applicable to
23 receptor ligand binding where the drug molecule binding to
24 the receptor with the greatest affinity usually has the
25 highest probability for initiating or inhibiting a signal.
26 However, this may not always be the case since it is possible

1 that in certain situations there may be cases where the
2 initiation or inhibition of a signal may be mediated through
3 non-receptor binding. The information conveyed by a
4 conformational change induced by ligand binding can have many
5 consequences such as a signal transduction, endocytosis,
6 among the others. The ability to produce a conformational
7 change in a receptor molecule may not necessarily be due to
8 the filling of a ligand receptor pocket but may occur through
9 the binding of another extra cellular domain or due to
10 receptor clustering induced by a multivalent ligand.

11 The production of antibodies to produce cell killing
12 need not be predicated upon screening of the hybridomas for
13 the best binding antibodies. Rather, although not advocated
14 by those who produce monoclonal antibodies, the screening of
15 the hybridoma supernatants for cell killing or alternatively
16 for cessation of growth of the cancerous cells may be
17 selected as a desirable endpoint for the production of
18 cytotoxic or cytostatic antibodies. It is well understood
19 that the *in-vivo* antibodies mediate their function through
20 the Fc portions and that the utility of the therapeutic
21 antibody is determined by the functionality of the constant
22 region or attached moieties. In this case the FAb portion of
23 the antibody, the antigen-combining portion, will confer to
24 the antibody its specificity and the Fc portion its
25 functionality. The antigen combining site of the antibody

1 can be considered to be the product of a natural
2 combinatorial library. The result of the rearrangement of the
3 variable region of the antibody can be considered a molecular
4 combinatorial library where the output is a peptide.
5 Therefore, the sampling of this combinatorial library can be
6 based on any parameter. Like sampling a natural compound
7 library for antibiotics, it is possible to sample an antibody
8 library for cytotoxic or cytostatic compounds.

9 The various endpoints in a screen must be differentiated
10 from each other. For example, the difference between antibody
11 binding to the cell is distinct from cell killing. Cell
12 killing (cytotoxicity) is distinct from the mechanisms of
13 cell death such as oncosis or apoptosis. There can be many
14 processes by which cell death is achieved and some of these
15 can lead either to oncosis or apoptosis. There is speculation
16 that there are other cell death mechanisms other than oncosis
17 or apoptosis but regardless of how the cell arrives at death
18 there are some commonalities of cell death. One of these is
19 the absence of metabolism and another is the denaturation of
20 enzymes. In either case vital stains will fail to stain these
21 cells. These endpoints of cell death have been long
22 understood and predate the current understanding of the
23 mechanisms of cell death. Furthermore, there is the
24 distinction between cytotoxic effects where cells are killed

1 and cytostatic effects where the proliferation of cells are
2 inhibited.

3 In a preferred embodiment of the present invention, the
4 assay is conducted by focusing on cytotoxic activity toward
5 cancerous cells as an end point. In a preferred embodiment,
6 a live /dead assay kit , for example the LIVE/DEAD
7 Viability/Cytotoxicity Assay Kit (L-3224) by Molecular
8 Probes, is utilized. The Molecular Probes kit provides a
9 two-color fluorescence cell viability assay that is based on
10 the simultaneous determination of live and dead cells with
11 two probes that measure two recognized parameters of cell
12 viability - intracellular esterase activity and plasma
13 membrane integrity. The assay principles are general and
14 applicable to most eukaryotic cell types, including adherent
15 cells and certain tissues, but not to bacteria or yeast.
16 This fluorescence-based method of assessing cell viability is
17 preferred in place of such assays as trypan blue exclusion,
18 Cr release and similar methods for determining cell viability
19 and cytotoxicity.

20 In carrying out the assay, live cells are distinguished
21 by the presence of ubiquitous intracellular esterase
22 activity, determined by the enzymatic conversion of the
23 virtually nonfluorescent cell-permeant CALCEIN AM to the
24 intensely fluorescent Calcein. The polyanionic dye Calcein
25 is well retained within live cells, producing an intense

uniform green fluorescence in live cells (ex/em ~495 nm/~515 nm). EthD-1 enters cells with damaged membranes and undergoes a 40-fold enhancement of fluorescence upon binding to nucleic acids, thereby producing a bright red fluorescence in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is excluded by the intact plasma membrane of live cells. The determination of cell viability depends on these physical and biochemical properties of cells. Cytotoxic events that do not affect these cell properties may not be accurately assessed using this method. Background fluorescence levels are inherently low with this assay technique because the dyes are virtually nonfluorescent before interacting with cells.

In addition to the various endpoints for screening, there are two other major characteristics of the screening process. The library of antibody gene products is not a random library but is the product of a biasing procedure. In the examples below, the biasing is produced by immunizing mice with fixed cells. This increases the proportion of antibodies that have the potential to bind the target antigen. Although immunization is thought of as a way to produce higher affinity antibodies (affinity maturation) in this case it is not. Rather, it can be considered as a way to shift the set of antigen combining sites towards the targets. This is also distinct from the concept of isotype switching where the functionality, as dictated by the

1 constant portion of the heavy chain, is altered from the
2 initial IgM isotype to another isotype such as IgG.

3 The third key feature that is crucial in the screening
4 process is the use of multitarget screening. To a certain
5 extent specificity is related to affinity. An example of this
6 is the situation where an antigen has very limited tissue
7 distribution and the affinity of the antibody is a key
8 determinant of the specificity of the antibody-the higher the
9 affinity the more tissue specific the antibody and likewise
10 an antibody with low affinity may bind to tissues other than
11 the one of interest. Therefore, to address the specificity
12 issue the antibodies are screened simultaneously against a
13 variety of cells. In the examples below the hybridoma
14 supernatants (representing the earliest stages of monoclonal
15 antibody development), are tested against a number of cell
16 lines to establish specificity as well as activity.

17 The antibodies are designed for therapeutic treatment of
18 cancer in patients. Ideally the antibodies can be naked
19 antibodies. They can also be conjugated to toxins. They can
20 be used to target other molecules to the cancer. e.g. biotin
21 conjugated enzymes. Radioactive compounds can also be used
22 for conjugation.

1 The antibodies can be fragmented and rearranged
2 molecularly: For example Fv fragments can be made; sFv-single
3 chain Fv fragments; diabodies etc.

4 It is envisioned that these antibodies can be used for
5 diagnosis, prognosis, and monitoring of cancer. For example
6 the patients can have blood samples drawn for shed tumor
7 antigens which can be detected by these antibodies in
8 different formats such as ELISA assays, rapid test panel
9 formats etc. The antibodies can be used to stain tumor
10 biopsies for the purposes of diagnosis. In addition a panel
11 of therapeutic antibodies can be used to test patient samples
12 to determine if there are any suitable antibodies for
13 therapeutic use.

14 The hybridoma cell lines 1LN-8 (shown in the table on
15 page 31), 3BD-8 (shown in the table on page 27), 3BD-26
16 (shown in the table on page 27), 3BD-27 (shown in the table
17 on page 27), H460-27 (shown in the table on page 46), H460-23
18 (shown in the table on page 46), 7BD-14 (shown in the table
19 on page 36) and 5LAC20 (shown in the table on page 42) were
20 deposited, in accordance with the Budapest Treaty, with the
21 American Type Culture Collection (ATCC), 10801 University
22 Blvd., Manassas, VA 20110-2209 on November 21, 2000 under
23 Accession Numbers PTA-2693, PTA-2696, PTA-2695, PTA-2698,
24 PTA-2699, PTA-2700, PTA-2697 and PTA-2694 respectively. The
25 hybridoma cell lines H460-16-2 (shown in the table on page

1 46) and 7BDI-60 (shown in the table on page 36) were deposited
2 on September 4, 2002 under Accession Numbers PTA-4621 and
3 PTA-4623 respectively. In accordance with 37 CFR 1.808, the
4 depositors assure that all restrictions imposed on the
5 availability to the public of the deposited materials will be
6 irrevocably removed upon the granting of a patent. The
7 depositors additionally assure that the deposited materials
8 will be replaced if viable samples cannot be dispensed by the
9 depository.

10 EXAMPLE ONE

11 In order to produce monoclonal antibodies specific for a
12 tumor sample the method of selection of the appropriate
13 hybridoma wells is complicated by the probability of
14 selecting wells which will produce false positive signals.
15 That is to say that there is the likelihood of producing
16 antibodies that can react against normal cells as well as
17 cancer cells. To obviate this possibility one strategy is to
18 mask the anti-normal antigen antibodies from the selection
19 process. This can be accomplished by removing the anti-
20 normal antibodies at the first stage of screening thereby
21 revealing the presence of the desired antibodies. Subsequent
22 limiting dilution cloning can delineate the clones that will
23 not produce killing of control cells but will produce target
24 cancer cell killing.

1 Biopsy specimens of breast, melanoma, and lung tumors
2 were obtained and stored at -70°C until used. Single cell
3 suspensions were prepared and fixed with -30°C, 70% ethanol,
4 washed with PBS and reconstituted to an appropriate volume
5 for injection. Balb/c mice were immunized with 2.5×10^5 - 1×10^6
6 cells and boosted every third week until a final pre-fusion
7 boost was performed three days prior to the splenectomy. The
8 hybridomas were prepared by fusing the isolated splenocytes
9 with Sp2/0 and NS1 myeloma partners. The supernatants from
10 the fusions were tested for subcloning of the hybridomas.

11 Cells (including A2058 melanoma cells, CCD-12CoN fibroblasts,
12 MCF-12A breast cells among others) were obtained from ATCC
13 and cultured according to enclosed instructions. The HEY cell
14 line was a gift from Dr. Inka Brockhausen. The non-cancer
15 cells, e.g. CCD-12CoN fibroblasts and MCF-12A breast cells,
16 were plated into 96-well microtitre plates (NUNC) 1 to 2
17 weeks prior to screening. The cancer cells, e.g. HEY, A2058,
18 BT 483, and HS294t, were plated two or three days prior to
19 screening.

20 The plated normal cells were fixed prior to use. The
21 plates were washed with 100 microliters of PBS for 10 minutes
22 at room temperature and then aspirated dry. 75 microliters
23 of 0.01 percent glutaraldehyde diluted in PBS were added to
24 each well for five minutes and then aspirated. The plates
25 were washed with 100 microliters of PBS three times at room

1 temperature. The wells were emptied and 100 microliters of
2 one percent human serum albumin in PBS was added to each well
3 for one hour at room temperature. The plates were then
4 stored at four degrees Celsius.

5 Prior to the transfer of the supernatant from the
6 hybridoma plates the fixed normal cells were washed three
7 times with 100 microliters of PBS at room temperature. After
8 aspiration to the microliters of the primary hybridoma
9 culture supernatants were transferred to the fixed cell
10 plates and incubated for two hours at 37 degrees Celsius in a
11 8 percent CO₂ incubator. The hybridoma supernatants derived
12 from melanoma was incubated with CCD-12 CoN cells and those
13 derived from breast cancer were incubated with MCF-12a cells.

14 After incubation the absorbed supernatant was divided
15 into two 75 microliter portions and transferred to target
16 cancer cell plates. Prior to the transfer the cancer cell
17 plates were washed three times with 100 microliters of PBS.
18 The supernatant from the CCD-12 CoN cells were transferred to
19 the A2058 and the HS294t cells, whereas the supernatant from
20 MCF-12A cells were transferred to the HEY and BT 483 cells.
21 The cancer cells were incubated with the hybridoma
22 supernatants for 18 hours at 37 degrees Celsius in an 8
23 percent CO₂ incubator.

24 The LIVE/DEAD cytotoxicity assay was obtained from
25 Molecular Probes (Eu,OR). The assays were performed according

1 to the manufacturer's instructions with the changes outlined
2 below. The plates with the cells were washed once with 100
3 microliters of PBS at 37°C. 75 to 100 microliters of
4 supernatant from the hybridoma microtitre plates were
5 transferred to the cell plates and incubated in a 8% CO₂
6 incubator for 18-24 hours. Then, the wells that served as the
7 all dead control were aspirated until empty and 50
8 microliters of 70% ethanol was added. The plate was then
9 emptied by inverting and blotted dry. Room temperature PBS
10 was dispensed into each well from a multichannel squeeze
11 bottle, tapped three times, emptied by inversion and then
12 blotted dry. 50 microliters of the fluorescent LIVE/DEAD dye
13 diluted in PBS was added to each well and incubated at 37°C
14 in a 5% CO₂ incubator for one hour. The plates were read in a
15 Perkin-Elmer HTS7000 fluorescence plate reader and the data
16 was analyzed in Microsoft Excel.

17 Four rounds of screening were conducted to produce
18 single clone hybridoma cultures. For two rounds of screening
19 the hybridoma supernatants were tested only against the
20 cancer cells. In the last round of screening the supernatant
21 was tested against a number of non-cancer cells as well as
22 the target cells indicated in table 1. The antibodies were
23 isotyped using a commercial isotyping kit.

24 A number of monoclonal antibodies were produced in
25 accordance with the method of the present invention. These

1 antibodies, whose characteristics are summarized in Table 1,
2 are identified as 3BD-3, 3BD-6, 3BD-8, 3BD-9, 3BD-15, 3BD-25,
3 3BD-26 and 3BD-27. Each of the designated antibodies is
4 produced by a hybridoma cell line deposited with the American
5 Type Culture Collection at 10801 University Boulevard,
6 Manassas, Va. having an ATCC Accession Number as follows:

7	<u>Antibody</u>	<u>ATCC Accession Number</u>
8	3BD-3	not deposited
9	3BD-6	not deposited
10	3BD-8	PTA-2696
11	3BD-9	not deposited
12	3BD-15	not deposited
13	3BD-25	not deposited
14	3BD-26	PTA-2695
15	3BD-27	PTA-2698

16 These antibodies are considered monoclonal after four rounds
17 of limiting dilution cloning. The anti-melanoma antibodies
18 did not produce significant cancer cell killing. The panel of
19 anti-breast cancer antibodies killed 32-87% of the target
20 cells and <1-3% of the control cells. The predominant isotype
21 was IgG1 even though it was expected that the majority of
22 anti-tumor antibodies would be directed against carbohydrate
23 antigens, and thus, be of the IgM type. There is a high

therapeutic index since most antibodies spare the control cells from cell death.

Table 1. Anti-Breast Cancer Antibodies

Clones	% Cell Death			
	Target for Anti-Breast Cancer Antibodies (HEY & A2058)	Normal Fibroblast Cells (CCD-12CoN)	Fibrocystic Breast Cells (MCF-12A)	Isotype
3BD-3	74.9%	3.7%	<1%	$\gamma 1, \lambda$
3BD-6	68.5%	5.6%	<1%	$\gamma 1, \lambda$
3BD-8	81.9%	4.5%	2.6%	$\gamma 1, \kappa$
3BD-9	77.2%	7.9%	<1%	$\gamma 1, \lambda$
3BD-15	87.1%	<1 %	<1%	$\gamma 1, \lambda$
3BD-26	54.8%	3.3%	<1%	μ, κ
3BD-25	32.4%	3.6%	<1 %	$\gamma 1, \kappa$
3BD-27	60.1%	8.3%	1.3%	$\gamma 1, \kappa$

EXAMPLE 2

In this example customized anti-cancer antibodies are produced by first obtaining samples of the patient's tumor. Usually this is from a biopsy specimen from a solid tumor or a blood sample from hematogenous tumors. The samples are prepared into single cell suspensions and fixed for injection into mice. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety of cancer cell

1 lines and normal cells in standard cytotoxicity assays. Those
2 hybridomas that are reactive against cancer cell lines but
3 are not reactive against normal non-transformed cells are
4 selected for further propagation. Clones that were considered
5 positive were ones that selectively killed the cancer cells
6 but did not kill the non-transformed cells. The antibodies
7 are characterized for a large number of biochemical
8 parameters and then humanized for therapeutic use.

9 The melanoma tumor cells isolated and cell lines were
10 cultured as described in Example 1. Balb/c mice were
11 immunized according to the following schedule: 200,000 cells
12 s.c. and i.p. on day 0, then 200,000 cells were injected i.p.
13 on day 21, then 1,000,000 cells were injected on day 49, then
14 1,250,000 cells in Freund's Complete Adjuvant were injected
15 i.p. on day 107, and then 200,000 cells were injected on day
16 120 i.p. and then the mice were sacrificed on day 123. The
17 spleens were harvested and the splenocytes were divided into
18 two aliquots for fusion with Sp2/0 (1LN) or NS-1 (2LN)
19 myeloma partners using the methods outlined in example 1.

20 The screening was carried out 11 days after the fusion
21 against A2058 melanoma cells and CCD-12CoN fibroblasts. Each
22 pair of plates were washed with 100 microliters of room
23 temperature PBS and then aspirated to near dryness. Then 50
24 microliters of hybridoma supernatant was added to the same
25 wells on each of the two plates. The spent Sp2/0 supernatant

1 was added to the control wells at the same volume and the
2 plates were incubated for around 18 hours at 37 degrees
3 Celsius at a 8%CO₂, 98% relative humidity incubator. Then
4 each pair of plates were removed and in the positive control
5 wells 50 microliters of 70% ethanol was substituted for the
6 media for 4 seconds. The plates were then inverted and washed
7 with room temperature PBS once and dried. Then 50uL of
8 fluorescent LIVE/DEAD dye diluted in PBS (Molecular Probes
9 LIVE/DEAD Kit) was added for one hour and incubated at 37
10 degrees Celsius. The plates were then read in a Perkin-Elmer
11 fluorescent plate reader and the data analyzed using
12 Microsoft Excel. The wells that were considered positive were
13 subcloned and the same screening process was repeated 13 days
14 later and then 33 days later. The results of the last
15 screening is outlined in Table 2 below. A number of
16 monoclonal antibodies were produced in accordance with the
17 method of the present invention. These antibodies, whose
18 characteristics are summarized in Table 2, are identified as
19 1LN-1, 1LN-8, 1LN-12, 1LN-14, 2LN-21, 2LN-28, 2LN-29, 2LN-31,
20 2LN-33, 2LN-34 and 2LN-35. Each of the designated antibodies
21 is produced by a hybridoma cell line deposited with the
22 American Type Culture Collection at 10801 University
23 Boulevard, Manassas, Va. having an ATCC Accession Number as
24 follows:

Antibody	ATCC Accession Number
1LN-1	not deposited
1LN-8	PTA-2693
1LN-12	not deposited
1LN-14	not deposited
2LN-21	not deposited
2LN-28	not deposited
2LN-29	not deposited
2LN-31	not deposited
2LN-33	not deposited
2LN-34	not deposited
2LN-35	not deposited

Table 2, Anti-Melanoma Antibodies

Clones	% Cell Death	
	Target for Anti-Melanoma Antibodies (A2058)	Normal Fibroblast Cells (CCD-1 2CoN)
1LN-1	59.4%	<1 %
1LN-8	11.0%	5.0%
1LN-12	55.2%	1.4%
1LN-14	51.4%	<1%
2LN-21	72.0%	15.9%
2LN-28	66.6%	12.4%
2LN-29	78.2%	6.1%
2LN-31	100%	7.8%
2LN-33	94.2%	<1%

2LN-34	56.6%	11.2%
2LN-35	66.5%	6.6%

The table illustrates that clones from both the Sp2/0 and NS-1 fusions were able to produce antibodies that had a greater than 50% killing rate for cancerous cells and at the same time some of the clones were able to produce less than one percent killing of normal control fibroblasts.

EXAMPLE 3

In this example antibodies were produced to several different breast tumor samples following the method of Example 2 in order to demonstrate the generality of producing customized antibodies. Biopsy specimens of breast tumors were obtained and stored at -70°C until used as noted in Example.

1. Single cell suspensions were prepared for each specimen and fixed with -30°C, 70% ethanol, washed with PBS and reconstituted to an appropriate volume for injection.

Female, 7-8 week old, A strain, H-2^d haplotype Balb/c mice (Charles River Canada, St. Constant, QC, Can), were immunized with 2.5×10^5 - 1×10^6 cells and boosted every third week until a final pre-fusion boost was performed three days prior to the splenectomy. The hybridomas were prepared by fusing the isolated splenocytes with Sp2/0 myeloma partners. The supernatants from the fusions were tested for subcloning of the hybridomas.

1
2 Hs574.T breast ductal carcinoma cells, A2058 melanoma
3 cells, NCI-H460 human lung large cell carcinoma, NCI-H661
4 human lung large cell carcinoma, CCD-112CoN human colon
5 fibroblasts, CCD-27sk human skin fibroblasts, MCF-12A human
6 mammary epithelial cells, Hs574.mg human breast cells and
7 other cell lines, were obtained from ATCC and cultured
8 according to enclosed instructions. Both cancer and non-
9 cancer cells were plated three to four days prior to
10 screening.

11 The hybridomas were cultured for ten to twelve days
12 after fusion and observed under the microscope. When 20 to
13 25% of the wells were over 80% confluent, the hybridoma
14 supernatants were screened in a cytotoxicity assay. The
15 hybridoma supernatants were divided into two 75 microliter
16 portions; one portion was added to a target cancer cell plate
17 and another to a non-cancer cell plate. Prior to transfer of
18 hybridoma supernatants, the cell plates were washed three
19 times with 100 microliters of PBS. The supernatant from the
20 anti-breast cancer hybridomas were transferred to the Hs574.T
21 and the Hs574.mg cells, whereas the supernatant from the
22 anti-lung cancer hybridoma were transferred to the NCI-H460
23 and CCD-27SK cells. The cancer cells were incubated with the
24 hybridoma supernatants for 18 hours at 37 degrees Celsius in
25 an 8 percent CO₂ incubator.

1 The LIVE/DEAD cytotoxicity assay was obtained from
2 Molecular Probes (Eugene,OR). The assays were performed
3 according to the manufacturer's instructions with the changes
4 outlined below. The plates with the cells were washed once
5 with 100 microliters of PBS at 37°C. 75 to 100 microliters
6 of supernatant from the hybridoma microtitre plates were
7 transferred to the cell plates and incubated in a 8% CO₂
8 incubator for 18-24 hours. Then, the wells that served as the
9 dead control cells were aspirated until empty and 50
10 microliters of 70% ethanol was added. The plate was then
11 emptied by inverting and blotted dry. Room temperature PBS
12 was dispensed into each well from a multichannel squeeze
13 bottle, tapped three times, emptied by inversion and then
14 blotted dry. 50 microliters of the fluorescent LIVE/DEAD dye
15 diluted in PBS was added to each well and incubated at 37°C
16 in a 5% CO₂ incubator for one hour. The plates were read in a
17 Perkin-Elmer HTS7000 fluorescence plate reader and the data
18 was analyzed in Microsoft Excel (Microsoft, Redmond, WA).

19 Four rounds of screening were conducted to produce
20 single clone hybridoma cultures. For two rounds of screening
21 the hybridoma supernatants were tested only against the
22 cancer cells. In the last round of screening the supernatant
23 was tested against a number of non-cancer cells as well as
24 the target cells indicated in Table 3. The antibodies were

1 isotyped using a commercial isotyping kit (Roche,
2 Indianapolis, IN).

3 A number of monoclonal antibodies were produced in
4 accordance with the method of the present invention. These
5 antibodies, whose characteristics are summarized in Table 3,
6 are identified as 4BD-1, 4BD-3, 4BD-6, 4BD-9, 4BD-13, 4BD-18,
7 4BD-20, 4BD-25, 4BD-37, 4BD-32, 4BD-26, 4BD-27, 4BD-28, 4BD-
8 50, 6BD-1, 6BD-3, 6BD-5, 6BD-11, 6BD-25, 7BD-7, 7BD-12-1,
9 7BD-12-2, 7BD-13, 7BD-14, 7BD-19, 7BD-21, 7BD-24, 7BD-29,
10 7BD-30, 7BD-31, 7BDI-17, 7BDI-58, 7BDI-60 and 7BDI-62. Each
11 of the designated antibodies is produced by a hybridoma cell
12 line deposited with the American Type Culture Collection at
13 10801 University Boulevard, Manassas, Va. having an ATCC
14 Accession Number as follows:

15	<u>Antibody</u>	<u>ATCC Accession Number</u>
16	4BD-1	not deposited
17	4BD-3	not deposited
18	4BD-6	not deposited
19	4BD-9	not deposited
20	4BD-13	not deposited
21	4BD-18	not deposited
22	4BD-20	not deposited
23	4BD-25	not deposited
24	4BD-37	not deposited
25	4BD-32	not deposited

1	4BD-26	not deposited
2	4BD-27	not deposited
3	4BD-28	not deposited
4	4BD-50	not deposited
5	6BD-1	not deposited
6	6BD-3	not deposited
7	6BD-5	not deposited
8	6BD-11	not deposited
9	6BD-25	not deposited
10	7BD-7	not deposited
11	7BD-12-1	not deposited
12	7BD-12-2	not deposited
13	7BD-13	not deposited
14	7BD-14	PTA-2697
15	7BD-19	not deposited
16	7BD-21	not deposited
17	7BD-24	not deposited
18	7BD-29	not deposited
19	7BD-30	not deposited
20	7BD-31	not deposited
21	7BDI-17	not deposited
22	7BDI-58	not deposited
23	7BDI-60	PTA-4623
24	7BDI-62	not deposited

25 These antibodies are considered monoclonal after four
 26 rounds of limiting dilution cloning. The panel of anti-breast

1 cancer antibodies killed 15-79% of the target cells and <1-
2 31% of the control cells. The majority of anti-tumor
3 antibodies were IgM type, suggesting they could be directed
4 against carbohydrate antigens on the surface of tumor cells.
5 There is a high therapeutic index since most of the
6 antibodies do not cause the normal cells to undergo cell
7 death.

8 These monoclonal antibodies are characterized for a number
9 of immunological and biochemical parameters. A cell based
10 enzyme linked immunosorbent assay (ELISA) was established for
11 measuring the binding of the antibodies derived of each
12 clones to different cell lines. Cells were seeded and grown
13 on 96-well tissue culture plates. The plates were washed with
14 100 microliters of PBS. 100 microliters of cold 4 percent
15 paraformaldehyde in PBS were added to each well for ten
16 minutes and then aspirated. The plates were washed with PBS
17 using a multichannel squeeze bottle . The wells were emptied
18 and 100 microliters of blocking buffer (1 percent
19 hydrocasein, 0.1 percent geletin in 50mM Tris-HCl buffer, pH
20 9.3) was added to each well for one hour at room temperature.
21 The plates were washed three times with a buffer (0.05
22 percent Tween 20 in 10 mM PBS) at room temperature and then
23 stored at -30 degrees Celsius with 100 microliters of the
24 buffer. Prior to use the plates were thawed and the buffer
25 was aspirated from each well. 75 microliters of hybridoma

1 supernatant were added to each well and incubated for 60
2 minutes at room temperature. After the plates were washed
3 with PBS using a multichannel squeeze bottle, 50 microliters
4 of a combination of peroxidase conjugated goat anti-mouse IgG
5 and peroxidase conjugated donkey anti-mouse IgM (Jackson
6 ImmunoResearch Lab, Inc., West Grove, PA.) was added and
7 incubated for 30 minutes at room temperature. After the last
8 wash, 50 microliters of orthophenylene diamine (OPD) (Sigma,
9 St. Louis, MO) was added to each well and the optical density
10 was read at 492 nm on the HTS7000 plate reader after adding
11 equal volume of 1 N sulfuric acid. Different clones show
12 different profiles in binding to different cells (Table 3).
13 This indicates that they may target different cell surface
14 antigen and further suggests the variable distribution of
15 these antigen on the surface of cancer cells. Those binding
16 only to cancer cells but not to normal cells could identify
17 certain tumor-associated antigen.

18
19 Table 3. Anti-Breast Cancer Antibodies

Clones	Isotype	% Cell Death		Binding to cell lines				
		Hs574.T	Hs574.mg	Hs574.T	Hs574.mg	NCI-H460	CCD-27sk	A2058
6BD-1	μ, κ	38.2	5	0.8	0.5	0.6	0.3	ND*
6BD-3	μ, κ	79	12	0.35	0.25	0.24	0.14	ND
6BD-5	μ, κ	57.3	8	1.0	0.3	0.14	0.25	ND
6BD-11	μ, κ	52.3	11	0.15	0.1	0.17	0.1	ND
6BD-25	μ, κ	33.3	2	0.15	0.1	0.2	0.1	ND
4BD-26	μ, κ	27	1.8	0.5	ND	ND	<0.1	ND
4BD-27	μ, κ	19.6	<1	0.9	ND	ND	0.5	ND
4BD-28	μ, κ	26.4	<1	0.8	ND	ND	<0.1	ND
4BD-32	μ, κ	41.7	4	0.8	ND	ND	<0.1	ND
4BD-50	μ, κ	20	<1	0.8	ND	ND	<0.1	ND
4BD-1	μ, κ	23	31	0.6	ND	ND	<0.1	ND
4BD-3	μ, κ	29.7	8.2	1.7	ND	ND	0.1	ND
4BD-6	μ, κ	17	<1	0.9	ND	ND	<0.1	ND
4BD-9	μ, κ	15	<1	0.6	ND	ND	<0.1	ND
4BD-13	μ, κ	31	<1	1.2	ND	ND	<0.1	ND
4BD-18	μ, κ	23.3	2.4	0.7	ND	ND	0.12	ND
4BD-20	μ, κ	45	<1	0.95	ND	ND	<0.1	ND
4BD-25	μ, κ	26	14.16	1.8	ND	ND	0.1	ND
4BD-37	μ, κ	30	<1	0.8	ND	ND	<0.1	ND
7BD-7	μ, κ	24	3	0.8	0.3	1.4	0.26	ND
7BD-12-1	μ, κ	22	6	0.36	0.16	0.43	0.1	ND
7BD-12-2	μ, κ	31	2	0.2	0.2	0.2	0.2	0.2
7BD-13	μ, κ	29	12	0.1	0.15	0.2	0.1	0.2
7BD-14	μ, κ	32	13	0.4	0.4	0.6	0.3	0.5
7BD-19	μ, κ	20	4	1.3	0.4	0.43	0.2	ND
7BD-21	μ, κ	21	13	0.4	0.5	0.25	0.3	ND
7BD-24	μ, κ	32	15	0.3	0.1	0.14	0.15	ND
7BD-29	μ, κ	15	16	0.3	0.24	0.14	0.16	ND

7BD-30	μ, κ	23	13	0.34	0.24	0.2	0.16	ND
7BD-31	μ, κ	28	10	0.3	0.4	0.4	0.3	0.4
7BDI-17	μ, κ	23	<1	0.75	ND	ND	ND	ND
7BDI-58	γ1, κ	17.5	<1	0.77	ND	ND	ND	ND
7BDI-60	γ1, κ	15	<1	0.73	ND	ND	ND	ND
7BDI-62		15	5	0.55	ND	ND	ND	ND

*ND: not done.

EXAMPLE 4

In this example customized anti-cancer antibodies are produced to a lung cancer sample by first obtaining samples of the patient's tumor preparing single cell suspensions which are then fixed for injection into mice as noted in Example 1. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that are reactive against cancer cell lines but are not reactive against normal non-transformed cells are selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells.

The lung cancer cells were isolated and cell lines were cultured as described in Example 1. Female, 7-8 week old, A strain, H-2^d haplotype Balb/c mice (Charles River Canada, St. Constant, QC, Can), were immunized with human

1 lung cancer cells. The lung cancer cell suspensions were
2 emulsified in an equal volume of Freund's complete adjuvant
3 (FCA) for the first immunization and then in Freund's
4 incomplete adjuvant (FIA) for subsequent immunizations at 0,
5 21, 45 days. 5×10^5 cells were used to immunize each mouse
6 either through a subcutaneous or intra-peritoneal route.
7 Immunized mice were sacrificed 3-4 days after the final
8 immunization with human lung cancer cells at 148 days, given
9 intra-peritoneally, in PBS at pH 7.4. The spleens were
10 harvested and the splenocytes were divided into two aliquots
11 for fusion with Sp2/0 myeloma partners using the methods
12 outlined in Example 1.

13 The screening was carried out 10 days after the fusion
14 against NCI-H460 and/or NCI-H661 cells and CCD-27SK
15 fibroblasts. Each pair of plates were washed with 100
16 microliters of room temperature PBS and then aspirated to
17 near dryness. Then 75 microliters of hybridoma supernatant
18 was added per well on each of the two plates. The spent Sp2/0
19 supernatant was added to the control wells at the same volume
20 and the plates were incubated for around 18 hours at 37
21 degrees Celsius at a 8%CO₂, 98% relative humidity incubator.
22 Then each pair of plates was removed and in the positive
23 control wells 50 microliters of 70% ethanol was substituted
24 for the media for 4 seconds. The plates were then inverted
25 and washed with room temperature PBS once and dried. Then 50

1 microliters of fluorescent live/dead dye diluted in PBS
2 (Molecular Probes LIVE/DEAD Kit) was added for one hour and
3 incubated at 37 degrees Celsius. The plates were then read in
4 a Perkin-Elmer fluorescent plate reader and the data analyzed
5 using Microsoft Excel. The wells that were considered
6 positive were subcloned and the same screening process was
7 repeated 6 days later and then 13 days later. The result of
8 the last screening is outlined in Table 4 below. Antibodies
9 were characterized for binding to different cell lines with a
10 cellular ELISA according to the methods of Example 3. A
11 number of monoclonal antibodies were produced in accordance
12 with the method of the present invention. These antibodies,
13 whose characteristics are summarized in Table 4, are
14 identified as 5LAC2, 5LAC4, 5LAC20, and 5LAC23. Each of the
15 designated antibodies is produced by a hybridoma cell line
16 deposited with the American Type Culture Collection at 10801
17 University Boulevard, Manassas, Va. having an ATCC Accession
18 Number as follows:

<u>Antibody</u>	<u>ATCC Accession Number</u>
5LAC2	not deposited
5LAC4	not deposited
5LAC20	PTA-2694
5LAC23	not deposited

Table 4. Anti-Lung Cancer Antibodies

Clones	Isotype	% Cell Death					Binding to cell lines				
		Hs574.T	NCI-H460	NCI-H661	A2058	CCD-27sk	Hs574.T	Hs574.mg	NCI-H460	CCD-27sk	A2058
5LAC2	μ, κ	30	7	45.3	23	<1	0.2	0.2	0.26	0.2	0.2
5LAC4	μ, κ	21	11	20.5	23	3	0.7	0.9	1.7	0.8	0.9
5LAC20	μ, κ	23	7	66.4	24	3	0.5	0.2	0.6	0.2	0.2
		23	8	57.6	25	5	0.6	0.6	0.6	0.6	0.6

*ND: not done

The table illustrates that clones were able to produce antibodies that had a greater than 7-67% killing rate for cancerous cells and at the same time some of the clones were able to produce less than five percent killing of normal control fibroblasts.

EXAMPLE 5

In this example customized anti-cancer antibodies are produced to a patient's lung cancer cells, but cultured cells were used in the antibody development process to demonstrate the generality of the immunization process. The samples were prepared into single cell suspensions and fixed for injection into mice as noted in Example 1. After the completion of three rounds of immunization with cells derived directly from a patient's lung cancer, the mice were immunized twice with a human lung large cell carcinoma cell line (NCI-H460).

1 Hybridomas were produced from splenocytes and the
2 supernatants were screened against a variety of cancer cell
3 lines and normal cells in standard cytotoxicity assays. Those
4 hybridomas that were reactive against cancer cell lines but
5 were not reactive against normal non-transformed cells were
6 selected for further propagation. Clones that were considered
7 positive were ones that selectively killed the cancer cells
8 but did not kill the non-transformed cells. The antibodies
9 are characterized for a large number of biochemical
10 parameters and then humanized for therapeutic use.

11 The lung tumor cells isolated and cell lines were
12 cultured as described in Example 1. Balb/c mice, A strain
13 with H-2^d haplotype from Charles River Canada, St. Constant,
14 Quebec, Canada, female, 7-8 week old, were immunized with the
15 human lung cancer cells emulsified in an equal volume of
16 either Freund's complete adjuvant (FCA) for the first
17 immunization and then in Freund's incomplete adjuvant (FIA)
18 for subsequent immunizations at 0, 21, 45 days with 5×10^5
19 cells. The mice were immunized with fixed NCI H460 cells,
20 which were prepared from NCI H460 cells grown in T-75 cell
21 culture flask by scraping mono-layer cells into cell
22 suspensions at 105, 150 and 170 days. Immunized mice were
23 sacrificed 3-4 days after the final immunization with NCI
24 H460 cells, given intra-peritoneally, in phosphate buffered
25 saline buffer (PBS), pH 7.4. The spleens were harvested and

1 the splenocytes were divided into two aliquots for fusion
2 with Sp2/0 myeloma partners using the methods outlined in
3 Example 1.

4 The screening was carried out 10 days after the fusion
5 against NCI H460 cells and CCD-27SK fibroblasts as described
6 in Example 4. Antibodies were characterized for binding to
7 different cell lines with a cellular ELISA according to the
8 methods of Example 3.

9 The wells that were considered positive were subcloned
10 and the same screening process was repeated 9 days and 18
11 days later. The results are outlined in Table 5 below. A
12 number of monoclonal antibodies were produced in accordance
13 with the method of the present invention. These antibodies,
14 whose characteristics are summarized in Table 5, are
15 identified as H460-1, H460-4, H460-5, H460-10, H460-14, H460-
16 16-1, H460-16-2, H460-23 and H460-27. Each of the designated
17 antibodies is produced by a hybridoma cell line deposited
18 with the American Type Culture Collection at 10801 University
19 Boulevard, Manassas, Va. having an ATCC Accession Number as
20 follows:

21	<u>Antibody</u>	<u>ATCC Accession Number</u>
22	H460-1	not deposited
23	H460-4	not deposited
24	H460-5	not deposited

1 H460-10 not deposited

2 H460-14 not deposited

3 H460-16-1 not deposited

4 H460-16-2 PTA-4621

5 H460-23 PTA-2700

6 H460-27 PTA-2699

7 Table 5. Anti-Lung Cancer Antibodies

Clones	isotype	% Cell Death				Binding to cell lines				
		NCI-H460	Hs574.T	A2058	CCD-	Hs574.	Hs574.m	NCI-	CCD-	A2058
H460-1	$\gamma 1, \hat{e}$	16	30	23	<1	1.0	0.6	0.5	0.7	ND
H460-4	$\gamma 1, \hat{e}$	37	21	23	3	1.0	0.6	0.4	0.6	ND
H460-5	μ, κ	22.5	23	24	3	1.0	0.3	0.3	0.2	ND
H460-10	μ, κ	8	23	25	5	0.3	0.14	0.2	0.1	ND
H460-14	$\gamma 1, \hat{e}$	17	ND	ND	4	1.1	0.6	0.4	0.54	ND
H460-16-1	$\gamma 1, \hat{e}$	33	ND	ND	8	1.0	0.6	0.3	0.5	ND
H460-16-2	$\gamma 1, \hat{e}$	22	ND	ND	3	1.0	0.6	0.3	0.7	ND
H460-22-1	$\gamma 1, \hat{e}$	21	ND	ND	5	0.6	0.4	0.3	0.4	ND
H460-22-2	μ, κ	23	ND	ND	3	0.4	0.1	0.1	0.1	ND
H460-23	μ, κ	36	36	18	1	0.4	1.1	0.54	0.53	0.58
H460-27	μ, κ	33	31	16	8	0.3	0.4	0.4	0.3	0.4

22 *ND: not done

23 The table illustrates that clones were able to produce

24 antibodies that had a greater than 15% killing rate for

25 cancerous cells and at the same time some of the clones were

1 able to produce less than eight percent killing of normal
2 control fibroblasts.

3 The anti-cancer antibodies of the invention are useful
4 for treating a patient with a cancerous disease when
5 administered in admixture with a pharmaceutically acceptable
6 adjuvant, for example normal saline, a lipid emulsion,
7 albumen, phosphate buffered saline or the like and are
8 administered in an amount effective to mediate treatment of
9 said cancerous disease, for example with a range of about 1
10 microgram per milliliter to about 1 gram per milliliter.

11 The method for treating a patient suffering from a
12 cancerous disease may further include the use of conjugated
13 anti-cancer antibodies and would this include conjugating
14 patient specific anti-cancer antibodies with a member
15 selected from the group consisting of toxins, enzymes,
16 radioactive compounds, and hematogenous cells; and
17 administering these conjugated antibodies to the patient;
18 wherein said anti-cancer antibodies are administered in
19 admixture with a pharmaceutically acceptable adjuvant, for
20 example normal saline, a lipid emulsion, albumen, phosphate
21 buffered saline or the like and are administered in an amount
22 effective to mediate treatment of said cancerous disease, for
23 example with a range of about 1 microgram per mil to about 1
24 gram per mil. In a particular embodiment, the anti-cancer

1 antibodies useful in either of the above outlined methods may
2 be a humanized antibody.

3
4 The anti-cancer antibodies of the invention are useful
5 for treating a patient with a cancerous disease when
6 administered in admixture with a pharmaceutically acceptable
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